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Synthesis and pH-dependent self-assembly of semifluorinated calix[4]arenes

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Abstract—A new series of highly fluorinated calix[4]arene-based amphiphilic molecules was designed and synthesized. Using the calix[4]arene scaffold, four perfluorinated hyper-hydrophobic groups and four water solubilizing chains were introduced in the same molecule and also segregated in space following the scaffold directionality. Upon solubilization in aqueous solutions, these amphiphilic molecules form microscopic fluorous domains that drive the formation of various self-assembly patterns. We found that the self-assembly of these semifluorinated calix[4]arenes is dependent on external stimuli, such as changes in the polarity of the solvent or pH. As a consequence, by changing the pH of the solutions, it is possible to shift the aggregation pattern of these molecules, by a regular change either in the shape or in the size of the initially formed ordered aggregates. These are examples of the variety of structures and possibilities in nano-engineering offered by fluorous-phase driven molecular recognition.

1. Introduction

Fluorination can drastically affect the physical–chemical properties of a molecule. While even one fluorine atom can change the hydrogen bonding properties and the lipophilicity of a molecule, the introduction of several fluorine atoms tends to generate a new behavior, performance, and potential for separation.[1] Perfluorination of organic molecules can generate a fluorous phase, which is at the origin of the unusual behavior of heavily fluorinated molecules and polymers.[2] Fluoro-organic compounds have interesting properties and are increasingly considered as potentially useful in various areas of life sciences, particularly in pharmaceutical research. The use of fluorinated building blocks in peptide, protein, and RNA design can lead to novel biomolecules with improved stability. Fluorinated colloidal systems are being investigated for the delivery of drugs, including oxygen, prodrugs, genes, vaccines, and other immunoactive agents, contrast media, and other materials.[3]

Due to their unique characteristics, fluorinated amphiphiles can self-assemble in various media into stable fluorinated colloids, generating organized nanometer-size fluorous phases.[4] Fluorinated surfactants display properties and performances that cannot be attained with standard surfactants. They are more surface-active, displaying critical micelle concentration (CMC) values that are usually two orders of magnitude lower than those of the hydrocarbon analogues.

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Fundamental knowledge about the nature and the origin of the fluorous phase can be gained by assessing the influence of multi-chain fluorinated amphiphiles on the resulting aggregate architecture. The unique combination of hydrophobicity and lipophobicity of fluorinated chains, together with the versatility of calix[4]arenes as molecular scaffolds can induce a novel structural compartmentalization and generate amphiphilic materials with new properties. We explored the application of the fluorophobic effect in molecular recognition by using macrocyclic molecules functionalized with perfluoroalkyl chains.[5] We designed a series of semifluorinated calix[4]arenes that self-assemble in organic and aqueous environments, forming aggregates with different morphologies. The self-assembly process is driven by the fluorophobic effect.[6] The three-dimensional, concave, and relatively rigid structure of the calixarenes, coupled with the well-developed synthetic methodologies, made these compounds attractive platforms for development of new supramolecular entities.[7] A variety of self-assembled systems based on the versatile calixarene scaffold have been studied, from dimeric capsules[8], to mono- and multilayers[9].

By combining the properties of amphiphiles with those of thermotropic liquid crystals, an interesting combination between order and mobility was achieved, which provides control of the aggregate architectures. The first member of this series, the calix[4]arene tetraamine **1**, was found to self-assemble in regularly ordered patterns in solvents as different as water, chloroform, and perfluorohexane.[10] This molecule was also found to exhibit glassy liquid crystalline behavior at room temperature, therefore it proved that liquid crystalline

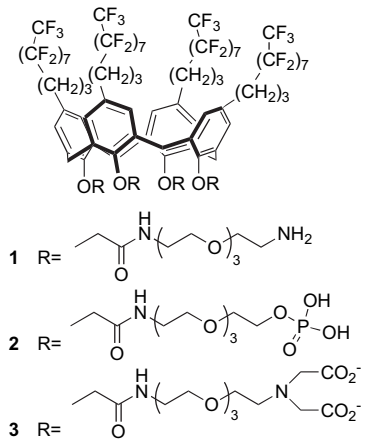


Figure 1. Chemical structure of the newly synthesized semifluorinated amphiphilic calix[4]arenes.

materials based on fluorinated calix[4]arenes can be designed by combining the bulky and stiff perfluorooctyl chains with the rigid calix[4]arene core.

This work has now been extended to include amphiphilic semifluorinated calix[4]arenes that are characterized by either a large number of negative charges to ensure higher aqueous solubility or functionalized with groups that are responsive to the solution pH. The new compounds that we have designed and synthesized are the calix[4]arene tetraphosphate **2** and the calix[4]arene octacarboxylate **3**. These compounds were designed to improve the aqueous solubility of these materials and to study the influence of pH on the self-assembly patterns. The solubility of calixarenes in water is very limited and in order to become water soluble, these compounds usually need to be functionalized with either groups containing charged functionalities or with neutral but highly hydrophilic moieties (Fig. 1).[11]

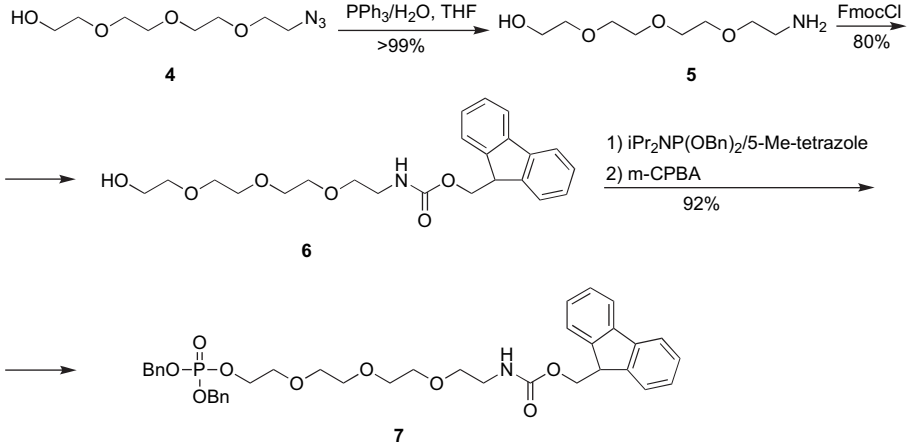
2. Results and discussion

2.1. Synthesis

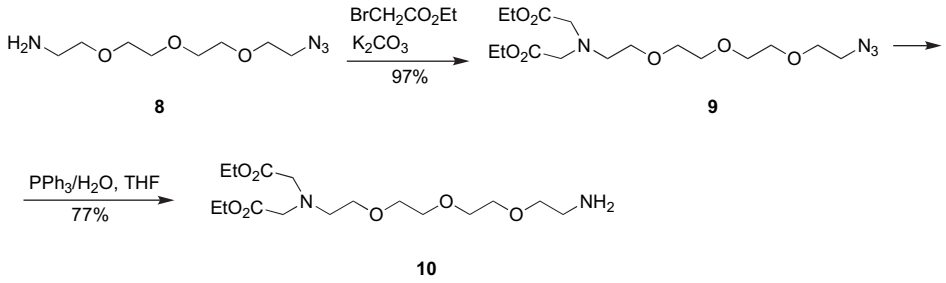
Calix[4]arene-based amphiphiles **2** and **3** were synthesized by attaching semifluorinated chains at the upper rim, and water solubilizing groups at the lower rim of the calix[4]arene scaffold. The hydrophilic part of the molecule is composed of a oligo(ethylene oxide) linker and a variously charged group. By grafting four highly fluorinated chains on the calix[4]arene scaffold, the amphiphilic character of these molecules is highly amplified. Formation of a fluorous phase in aqueous environments induces the self-assembly of these highly fluorinated molecules. Intercalation of a short alkyl chain between the calixarene scaffold and the rigid fluorous chains imparts increased flexibility to the fluorophilic moiety of the molecule, making possible the formation of several alternative self-assembled structures.

The modular design of the molecules allowed the synthesis of semifluorinated calix[4]arenes with different charged groups, such as free amines, phosphates, and aminodiacetates. The amine functionalities allow up to four positive charges on the molecules, while the phosphates can allow up to eight negative charges. The aminodiacetate group was chosen to study the effect of manipulating the number of charges by pH control. In this case, positively charged, negatively charged or zwitterionic molecules can be obtained, depending on the pH of the medium. We found that the resulting self-assembled structures are dependent on the pH of the solution as the number and nature of the charges on the external surface of the aggregates dictate their final shape and size.

The synthesis of the perfluorooctyl functionalized calix[4]arene and calix[4]arene tetraamine **1** were reported elsewhere.[5] The perfluorooctyl chains at the upper rim were introduced by a radical addition reaction to *p*-allyl-calix[4]arene with perfluorooctyl iodide, followed by hydrodeiodination. After installing the four carboxylate groups at the lower rim, the calix[4]arene tetracarboxylic acid was coupled with the corresponding ethylene glycol amine using standard peptide chemistry. A similar approach was used for the synthesis of the calix[4]arene tetraphosphate **2** and calix[4]arene octacarboxylate **3**. The corresponding oligo(ethylene oxide) amines were synthesized starting from known intermediates, as shown in Schemes 1 and 2. The 9-fluorenylmethyl (Fmoc) protecting group[12] was chosen, in light of its orthogonality with the benzylphosphate group[13]. The dibenzylphosphate group was introduced



Scheme 1. Synthesis of the benzylphosphate oligo(ethylene oxide) amine.



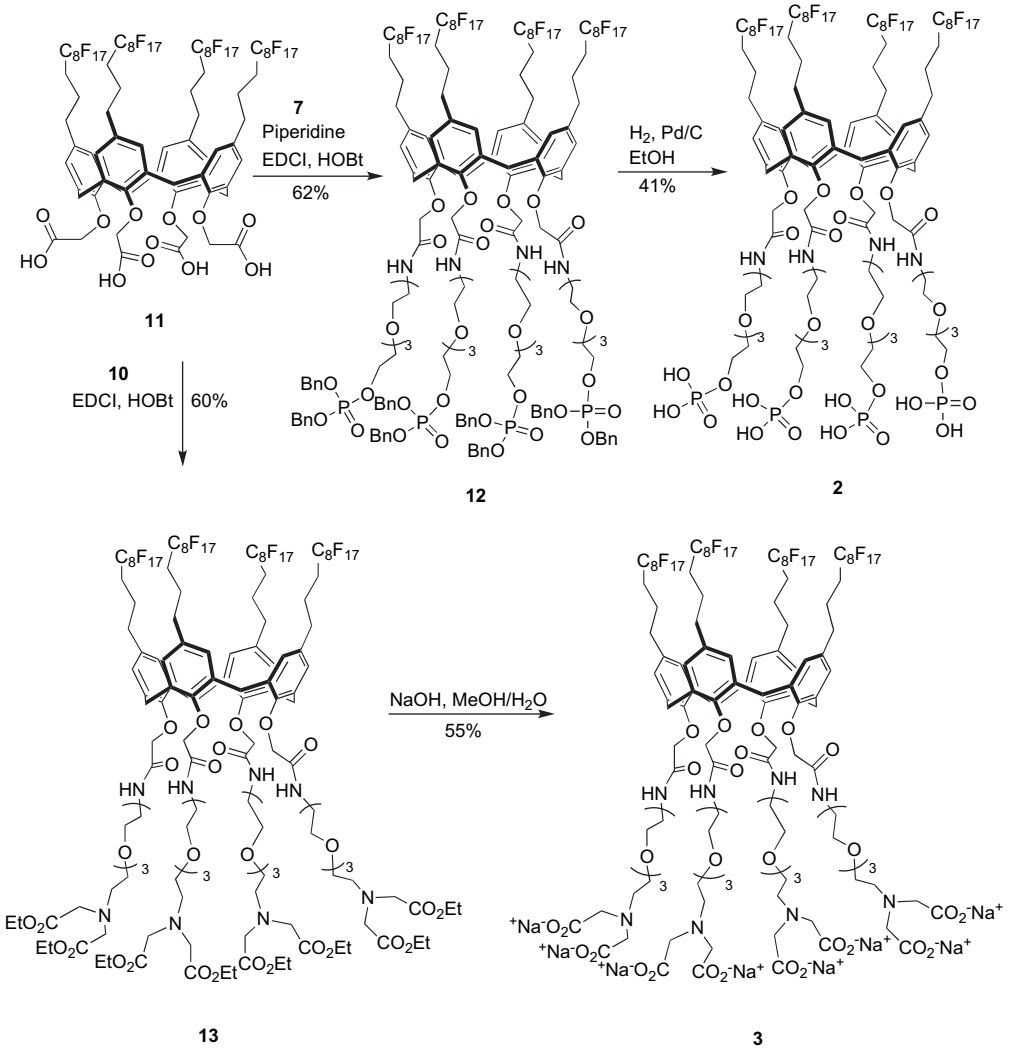
Scheme 2. Synthesis of the aminodiacetate-oligo(ethylene oxide) amine.

with dibenzyl diisopropyl phosphoramidite followed by oxidation[14], as shown in Scheme 1. The monoazide **4** was converted to the monoamine **5** by a Staudinger reduction with triphenylphosphine in quantitative yield.[15] The monoamine was further protected with Fmoc (80% yield) and the benzyl phosphate groups were introduced using diisopropyl dibenzyl phosphoramidite and 5-methyltetrazole, followed by *m*-CPBA oxidation with a 92% yield for two steps.

The aminodiacetate functionalized oligo(ethylene oxide) amine was synthesized by treating amino-azide **8** with ethyl bromoacetate.[16] The resulting azide was subjected to the Staudinger reduction conditions[17] to give 1-bis(ethoxycarbonylmethyl)amino-11-amino-3,6,9-trioxaundecane **10** in 75% overall yield.

Standard carbodiimide conditions with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBt) for amide bond formation, were used for coupling tetraacid **11** with the corresponding free amines (Scheme 3).

Deprotection of both the phosphate and aminodiacetate calix[4]arenes yielded the target amphiphilic semifluorinated



Scheme 3. Synthesis of the amphiphilic semifluorinated calix[4]arenes **2** and **3**.

calix[4]arenes **2** and **3**. The Fmoc-dibenzylphosphate amine **7** was deprotected with piperidine in dimethylformamide and then immediately added to the activated carboxylic acid without isolation, in the presence of the coupling reagents. Finally, the dibenzylphosphate groups in the protected product **12** were cleaved by hydrogenolysis.[18] The ethyl ester groups in compound **13** were subjected to base hydrolysis with sodium hydroxide to give the calix[4]arene octacarboxylate **3**.

2.2. Self-assembly studies

The self-assembly behavior of this new class of semifluorinated calix[4]arene amphiphiles was studied using a combination of dynamic light scattering (DLS) and transmission electron microscopy (TEM). The two calix[4]arene derivatives **2** and **3** were used to explore the influence of pH changes on the aggregate architecture. Both these molecules exhibit improved water solubility compared to the calix[4]-arene tetraamine **1**, which is a direct consequence of the higher number of charges on each molecule. The tetraphosphate **2** has eight negative charges in basic buffers, but still maintains four negative charges at pH 4.1, which allow for its dissolution in the acetate buffer. This behavior is a consequence of the pK_a values of a phosphate group, which are known to be 1.60 and 6.62 for ethyl phosphate.[19] Solutions of the above two calix[4]arene derivatives at different pHs were prepared by sonication of a thin film of compound **2** or **3** in the corresponding aqueous buffer. All the solutions had a concentration of 100 μM and were prepared in a similar manner for both the DLS and the TEM studies. The critical aggregation concentrations were not determined because even at the lowest possible concentrations detected by DLS, the presence of non-aggregated molecules was not detected. The solubility of the calix[4]arene octacarboxylate **3** does not change much with pH, while the solubility of the tetraphosphate **2** decreases slightly going from pH 12 to pH 4.1. This is correlated with a reduction in the number of negative charges on the molecules from eight to four.

The following buffer systems were prepared and used to dissolve the samples: hydrochloric acid buffer (pH 1.2), phtalate buffer (pH 3), acetate buffer (pH 4.1), phosphate buffers (pH 5.8, 7.4, and 12), and borate buffer (pH 8.6). The viscosities and refractive indexes for each buffer were measured separately and used as parameters for the DLS measurements. The population distributions obtained for the calix[4]arene tetraphosphate solutions are similar for all the buffers that were examined. Three distributions were determined for each solution. For example, at pH 4.1 three distributions were observed, centered at 24 nm (6% of the aggregates), 98 nm (45%), and 265 nm (49%). By analyzing the corresponding electron micrograph (Fig. 2), it appears that small spherical micelles are formed, with sizes of 15–20 nm, which further cluster together into larger aggregates, which represent the other population distributions in the DLS profiles. In basic buffers this negatively charged calix[4]arene derivative self-assembles into spherical micelles with an average diameter of 15–20 nm (Fig. 2a and b). These micelles can further cluster into larger aggregates.

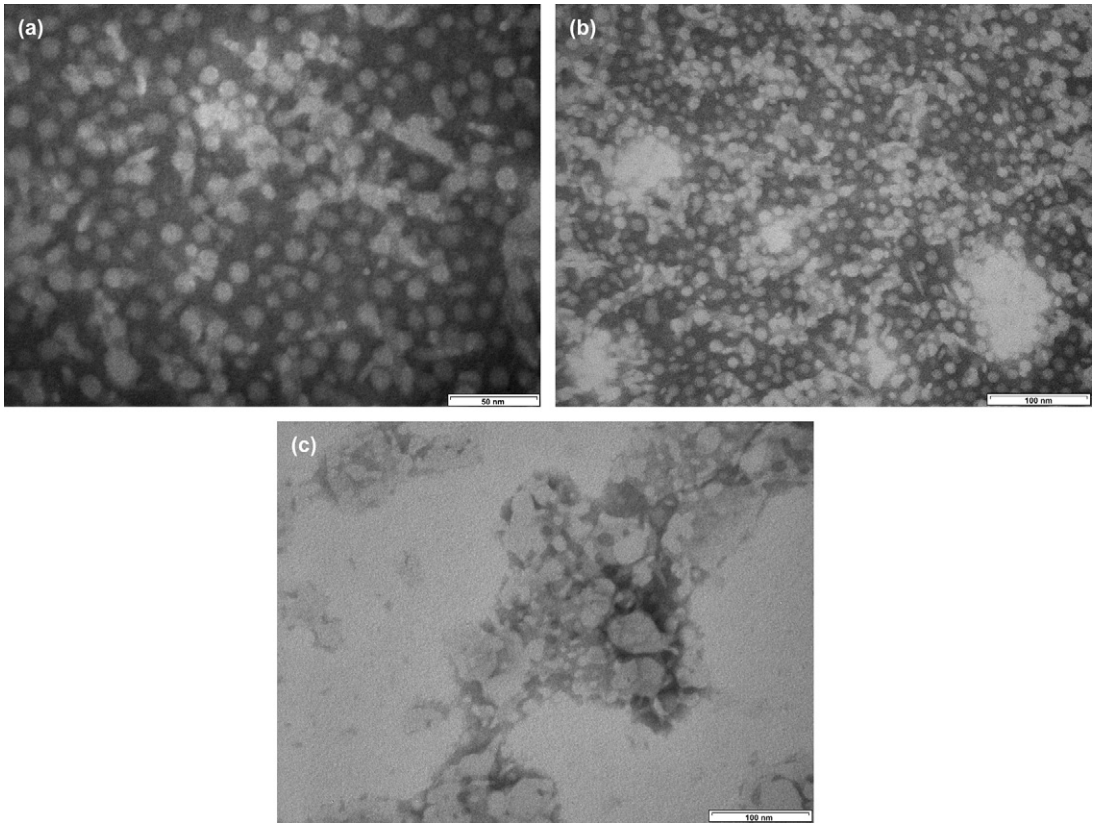


Figure 2. Transmission electron micrographs of calix[4]arene tetraphosphate [**2**]=100 μM. (a) Borate buffer of pH 8.6 (the scale bar represents 50 nm). (b) Borate buffer of pH 8.6 (the scale bar represents 100 nm). (c) Acetate buffer of pH 4.1 (the scale bar represents 100 nm).

When the number of charges is decreased to four, at pH 4.1, the aggregation behavior is less regular and the aggregates do not have a constant size or shape, as observed in Figure 2c.

Most of the sample at pH 4.1 is assembled into bilayer structures and a small fraction is still forming micelles and other small aggregates. Therefore, a decrease in the number of negative charges on the molecules leads, as expected, to a decreased ability to form spherical micelles for the calix[4]-arene tetraphosphate **2**. For the calix[4]arene octacarboxylate **3**, the patterns are more complex and more difficult to interpret due to the various charged states this molecule can adopt. The acidic properties of monomeric alkyl-*N*-iminodiacetic acids in water are known to be in the expected ranges with pK_a values of about 1.7, 2.3, and 10.3.[20] However, *n*-octadecyl-*N*-iminodiacetic acid that is present as aggregates in water, was found to display very acidic properties of the first proton and a substantially weakened acidity of the second proton (p$K_{a_2}=5.5-7.5$, depending on the ionic strength) and p$K_{a_3}=9.5-10.5$.[20] Based on these pK_a values determined for alkyl-*N*-iminodiacetic acids, the possible charged states of the calix[4]arene octacarboxylate are presented in Figure 3.

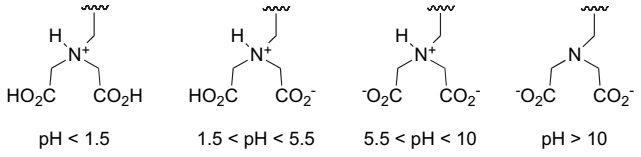


Figure 3. Various charged states of the iminodiacetic group in calix[4]arene octacarboxylate **3**.

This pattern of acidic constants suggests that a polymer structure with hydrogen bonds is maintained in the aggregates and that such bonds can exist in aqueous systems if they are supported by a strong and rigid backbone structure, as for example, the bilayers of well-organized long interdigitating alkyl chains.[20] This rigid structure also exists in the calix[4]arene octacarboxylate **3** and is formed by the combination of the calix[4]arene core and the bulky perfluorooctyl chains on the upper rim. The opposite part of the molecule, however, is more flexible due to the presence of the hydrated oligo(ethylene oxide) units, therefore this system is more complex than the *n*-alkyl-*N*-iminodicarboxylates. Based on the evidence that short strong hydrogen bonds form in the aggregates of long chain *n*-alkyl-*N*-iminodiacetic acids,[20] a similar behavior can be envisioned for the calix[4]octacarboxylate-based assemblies. The formation of these hydrogen bonds could explain the fluctuation in the size of the micellar aggregates with pH, as observed by TEM and DLS.

TEM analysis (Fig. 4) suggests that the octacarboxylate **3** exhibits a similar behavior to the tetraphosphate **2**, forming small spherical micelles, which cluster together into larger aggregates. The size of the majority of these micelles changes with the pH, from 17–20 nm at acidic pH (1.2, 3, and 4.1) to 40–60 nm at pH 8.6. This change in size can be correlated with a change in the charged state of the molecules.

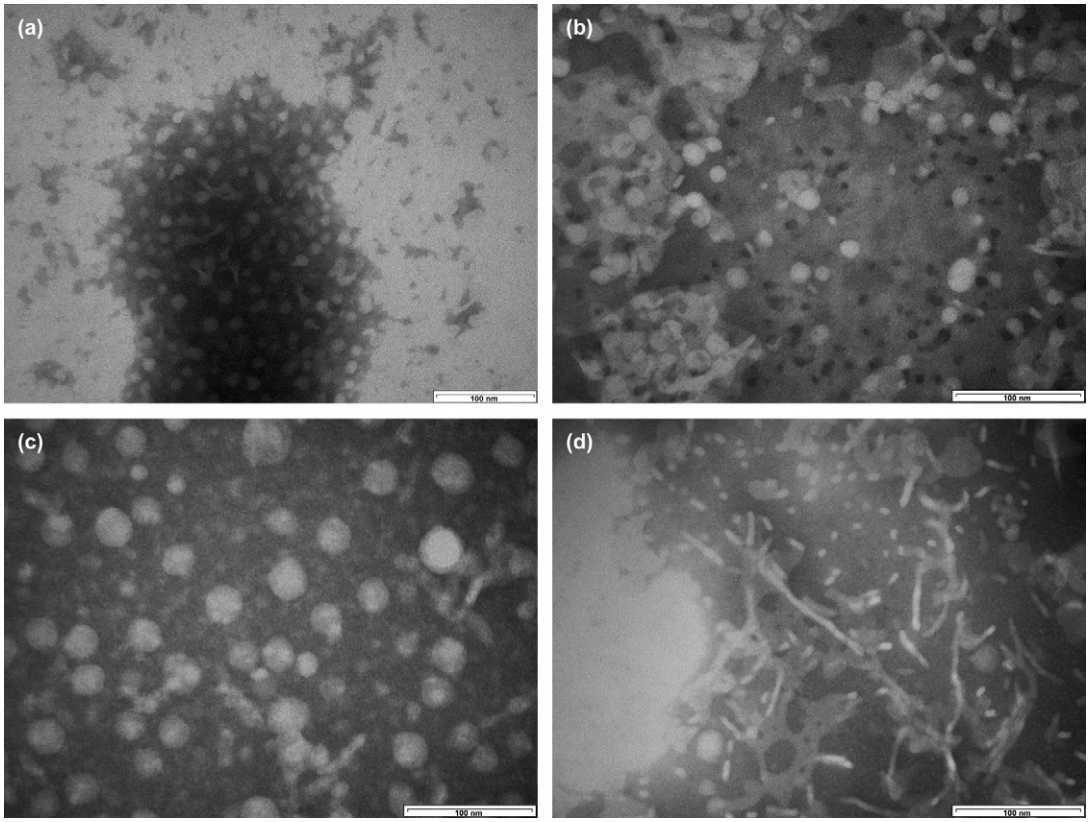


Figure 4. Transmission electron micrographs of calix[4]arene octacarboxylate [**3**]=100 μM, the scale bar represents 100 nm. (a) HCl buffer of pH 1.2. (b) Acetate buffer of pH 4.1. (c) Borate buffer of pH 8.6. (d) Phosphate buffer of pH 12.

With the increase in pH, the charged state of the molecule changes from four positive charges (at pH 1.2) to a zwitterionic molecule with overall neutral charge (at pH 3 and 4.1), to a total of four negative charges at pH 7.4 and 8.6. This determines an increase in the size of the micelles that are formed, probably due to the decreased ionic repulsion or an increased ionic attraction between the positive and negative charges of adjacent similarly charged functional groups. This explanation is consistent with the pK_a values published for the long chain *n*-alkyl-*N*-iminodiacetic acids. The second carboxylic acid proton participates in the hydrogen bonds and, as a consequence of this interaction, its pK_a is five times less than the analogue monomers with short alkyl chains (Fig. 3).[20] Furthermore, the pK_{a_2} value was found to be dependent on the ionic strength of the medium with the acidity increasing with increasing ionic strength. The acidic properties of the ammonium group did not seem to be much influenced by the aggregation behavior of the *n*-alkyl-*N*-iminodiacetic acids.[20]

A very important change in the morphology of the calix[4]arene octacarboxylate aggregates is the appearance of cylindrical micelles in a basic environment. At pH 12, these appear to be the majority of the smaller aggregates (Fig. 4d). In phosphate buffer at pH 12, a minor fraction of the sample is still self-assembled into spherical micelles and bilayers, but the main components of the mixture are the cylindrical micelles. Even at pH 8.6 there are a few cylindrically shaped aggregates present, meaning that the change is slow, starts at pH 8.6 and is not completed at pH 12. The overall influence of the pH is much stronger for the calix[4]arene octacarboxylate than for the other derivatives, due to the various charged states of this molecule.

The following trends were observed for the calix[4]arene octacarboxylate **3**: (1) the micelle size increases with increasing pH, (2) the shape of the micelles changes from spherical to cylindrical with increasing pH, and (3) bilayer formation is favored by extreme acidic and extreme basic pHs and less favored in a more neutral environment.

3. Conclusions

New semifluorinated amphiphiles composed of a calix[4]arene scaffold functionalized with linear fluorinated chains at the upper rim and hydrophilic groups at the lower rim were synthesized. Using the unique calix[4]arene scaffold, four fluorophilic (hyper-hydrophobic) groups and four water solubilizing chains can be introduced in the same molecule and also segregated in space by the scaffold directionality. The self-assembly properties of these novel semifluorinated calix[4]arenes were studied in solvents of varying polarity and different aqueous buffers. These molecules form microscopic fluorous domains that drive the formation of polarity and pH-dependent self-assembly patterns. These are examples of the variety of structures and possibilities offered by fluorous-phase driven recognition.

The calix[4]arene octacarboxylate **3** and calix[4]arene tetraphosphate **2** exhibit increased water solubility than calix[4]arene tetraammonium **1** and form a variety of aggregates depending on the pH of the medium. Two types of aggregates were consistently present in all the buffers: bilayers and micelles. The bilayers seem to remain unchanged and are more abundant at extremely acidic and extremely basic pHs, compared to the more neutral samples. The aggregates are held together by the fluorophobic effect and also by hydrogen bonds within the surfaces of the aggregates containing aminodiacetate groups. There seems to be a fine balance between the hydration of the aggregate and the formation of the fluorous phase with the perfluorooctyl chains. Due to the unusual strength of fluorophobic effect in polar environments, the hydration of the aggregate surfaces is not sufficiently strong to break these assemblies into monomers at any pH.

By changing the pH of the solutions, it is possible to shift the aggregation pattern of these molecules, for example, to determine an increase in the size of the micelles or to change their shape. These semifluorinated calix[4]arene materials respond to external stimuli, such as changes in the polarity of the solvent or pH. Therefore it is conceivable that solvent dependence of fluorous-phase driven self-assembly could be used in sensing devices and as a molecular tool to organize complex self-assembling structures. In conclusion, significant changes in the aggregate architecture with pH were observed for the two amphiphilic calix[4]arene derivatives **2** and **3**. Although these changes are not kinetically very fast and there is not a complete shift from one aggregation form to another, the properties of these molecules represent an important advance toward the design of smart nanomaterials that can respond to external stimuli.

4. Experimental section

4.1. General

4.1.1. 1-(Amino)-11-hydroxy-3,6,9-trioxaundecane, 5. A solution of 1-azido-11-hydroxy-3,6,9-trioxaundecane (5 g, 22.83 mmol) in 125 mL anhydrous tetrahydrofuran under an argon flow was cooled to 0 °C. Triphenylphosphine (6.59 g, 25.11 mmol) was added and the mixture was allowed to slowly warm to room temperature, after which was stirred for 2 d. A small volume of water (1 mL) was added to hydrolyze the intermediate phosphorus adduct. The reaction mixture was stirred at room temperature overnight and then at 40 °C for 6 h. After diluting with water, the solvent was removed in vacuo and 0.2 N HCl was added until pH 3 was reached. The suspension was washed three times with toluene and the aqueous solution was concentrated down to a colorless gum. Purification by flash chromatography (gradient from 0 to 20% methanol in dichloromethane) yielded 5 g (95%) product as a colorless oil. MS (ESI) *m/z* [M+H] calcd for $C_8H_{19}NO_4$ 194.1392, found 194.1390. ^{1}H NMR ($CDCl_3$) δ: 3.20–3.30 (br s, 2H), 3.6–4.0 (m, 12H), 4.38–4.41 (br s, 2H), 8.15–8.25 (br s, 2H). ^{13}C NMR ($CDCl_3$) δ: 40.1, 61.1, 67.1, 69.8, 70.0, 70.3, 70.4, 72.5.

4.1.2. 1-(Fluorenylmethoxycarbonylamino)-11-hydroxy-3,6,9-trioxaundecane, 6. To a solution of 1-amino-11-hydroxy-3,6,9-trioxaundecane (2.6 g, 11.3 mmol) in dioxane, 18 mL 10% aqueous sodium bicarbonate was added, followed by cooling to 0 °C. A solution of 3.3 g (12.46 mmol) FmocCl in dioxane was slowly added, followed by stirring

at 0 °C for 4 h and room temperature overnight. The reaction mixture was diluted with water and then extracted with dichloromethane. The Fmoc-protected amine was obtained as a yellow oil (4.1 g, 87% yield) after purification by flash chromatography using ethyl acetate/petroleum ether 2:1, followed by ethyl acetate. MALDI-FTMS: $[M+Na]^+$ calcd for $C_{23}H_{29}NO_6Na$ 438.20, found 438.188. ^1H NMR ($CDCl_3$) δ: 3.25–3.4 (m, 2H), 3.45–3.75 (m, 14H), 4.25 (t, 1H, J=6.8 Hz), 4.45 (d, 2H, J=6.8 Hz), 6.25 (br s, 1H), 7.25–7.35 (m, 2H), 7.38–7.42 (m, 2H), 7.63 (d, 2H, J=7.6 Hz), 7.75 (d, 2H, J=7.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 41.1, 47.6, 61.7, 66.6, 70.3, 70.6, 70.8, 72.8, 120.1, 125.3, 127.2, 127.8, 141.5, 144.3, 157.0.

4.1.3. 1-(Fluorenylmethoxycarbonylamino)-11-dibenzylphosphate-3,6,9-trioxaundecane, 7. To a stirred mixture of 1-(fluorenylmethoxycarbonylamino)-11-hydroxy-3,6,9-trioxaundecane (0.159 g, 0.24 mmol) and 5-methyltetrazole (0.04 g, 0.48 mmol) in anhydrous acetonitrile, was added dibenzyl diisopropyl phosphoramidite (0.095 mL, 0.29 mmol). The mixture was stirred at room temperature under argon for 3 h, and then poured into saturated aqueous sodium bicarbonate. After filtering the resulting white precipitate, the filtrate was diluted with ethyl acetate, washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give 209 mg yellow oil, which was dried under vacuum and used in the next step without purification. To an ice-cooled solution of the above phosphate intermediate in anhydrous dichloromethane 0.059 g (0.265 mmol) *m*-chloroperoxibenzoic acid was added and the stirring was continued at 0 °C for 1 h. The reaction mixture was then treated with saturated aqueous sodium thiosulfate and extracted with dichloromethane. The organic layer was successively washed with water, saturated sodium bicarbonate, and brine, before being dried over anhydrous magnesium sulfate. Purification by preparative TLC in ethyl acetate gave 150 mg colorless oil (92%). MALDI-FTMS: $[M+Na]^+$ calcd for $C_{37}H_{42}NO_2PNa$ 698.26, found 698.245. ^1H NMR ($CDCl_3$) δ: 3.25–3.4 (m, 2H), 3.45–3.75 (m, 12H), 4.15 (m, 2H), 4.25 (t, 1H, J=6.8 Hz), 4.45 (d, 2H, J=6.8 Hz), 5.0–5.1(m, 4H), 6.25 (br s, 1H), 7.25–7.35(m, 2H), 7.38–7.42 (m, 2H), 7.63 (d, 2H, J=7.6 Hz), 7.75 (d, 2H, J=7.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 41.2, 47.5, 66.8, 67.0, 69.4, 69.5, 70.1, 70.2, 70.3, 70.5, 70.8, 70.8, 120.2, 125.3, 127.3, 127.9, 128.2, 128.7, 128.8, 136.1, 141.5, 144.2.

4.1.4. 1-Bis(ethoxycarbonylmethyl)amino-11-azido-3,6,9-trioxaundecane, 9. To a solution of 1.55 g (7.11 mmol) 1-amino-11-azido-3,6,9-trioxaundecane in 50 mL anhydrous acetonitrile, 1.96 g (14 .2 mmol) anhydrous potassium carbonate was added and the suspension stirred at room temperature for 3 h before adding 1.6 mL (14.2 mmol) 98% ethyl bromoacetate. Stirring was continued at room temperature for 2 d. After removal of the solvent, the solid residue was partitioned between ethyl acetate and water. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and concentrated down to a yellow oil, which was purified by flash chromatography (ethyl acetate/petroleum ether 2:1) to give 2.23 g product (86%). ^1H NMR ($CDCl_3$) δ: 1.26 (t, 6H, J=7.2 Hz), 2.97 (t, 2H, J=5.6 Hz), 3.40 (t, 2H, J=5.3 Hz), 3.59–3.69 (m, 16H), 4.13–4.18 (q, 4H, J=7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.4, 50.8, 53.8, 56.0, 60.6, 70.2, 70.5, 70.8, 171.5.

4.1.5. 1-Bis(ethoxycarbonylmethyl)amino-11-amino-3,6,9-trioxaundecane, 10. A solution of 0.42 g (1.06 mmol) 1-bis(ethoxycarbonylmethyl)amino-11-azido-3,6,9-trioxaundecane in anhydrous tetrahydrofuran was cooled to 0 °C. Triphenylphosphine (0.31 g, 1.16 mmol) was added, after which the mixture was allowed to attain room temperature and was stirred for 2 d. After addition of 50 μL (2.8 mmol) water, the solution was stirred at room temperature for 24 h and then at 40 °C for 6 h. After diluting with water and removal of the tetrahydrofuran in vacuo, the pH was adjusted to 3 with 0.2 N HCl and the resulting suspension was washed with toluene. The aqueous solution was concentrated down to a colorless oil, which was purified by flash chromatography (10–20% methanol in dichloromethane) to yield 0.3 g product (77%). MS (ESI) m/z [M+H] calcd for $C_{16}H_{33}N_2O_7$ 365.2288, found 365.2283. ^1H NMR ($CDCl_3$) δ: 1.27 (t, 6H, J=7.2 Hz), 2.92 (t, 2H, J=5.6 Hz), 3.18 (m, 2H), 3.48–3.78 (m, 16H), 3.94 (m, 2H), 4.13–4.18 (q, 4H, J=7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.2, 40.5, 54.8, 55.3, 61.2, 67.1, 67.5, 69.8, 69.9, 70.1, 70.2, 171.4.

4.1.6. Calix[4]arene tetrabenzylphosphate, 12. To an anhydrous tetrahydrofuran solution of 5,11,17,23-tetra-(heptadecafluoro-undecyl)-25,26,27,28-calix[4]arene tetraacetic acid (100 mg, 0.04 mmol), 50.61 mg 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 35.67 mg (0.264 mmol) 1-hydroxybenzotriazole hydrate (HOBt) were added, followed by anhydrous dimethylformamide dropwise until all the solids dissolved. The reaction mixture was stirred at room temperature under an argon atmosphere for 4 h. Fmoc-amine **7** of 162.168 mg (0.24 mmol) was dissolved in 2 mL anhydrous dimethylformamide, 26 μL (22.5 mg, 0.264 mmol) piperidine was added, and the mixture was stirred at room temperature for 6 h, before being added to the first solution of activated acid. Stirring was continued for 7 d at room temperature. After removing all the solvents under an air stream, the residue was taken up in dichloromethane and washed with water, dried over anhydrous magnesium sulfate, and concentrated down to a yellow solid. Benzyl protected product of 106 mg was obtained (62% yield after flash chromatography). MALDI-FTMS $[M+Na]^+$ calcd for $C_{168}H_{172}F_{68}N_4O_{36}P_4Na$ 4259.96, found 4260.319. ^1H NMR ($CDCl_3$) δ: 1.65–1.8 (br m, 8H), 1.9–2.1 (br m, 8H), 2.2–2.4 (br m, 8H), 3.1–3.25 (d, 4H, J=8.5 Hz), 3.4–3.7 (m, 64H), 4.05–4.2 (m, 8H), 4.5–4.65 (d, 4H, J=8.5 Hz), 4.95–5.1 (m, 16H), 6.2–6.6 (br s, 8H), 7.25–7.3 (s, 40H), 7.9 (s, 4H). ^{19}F NMR ($CDCl_3$) δ: −81.5 (t, 12F), −114.5 (m, 8F), −122.5 to −122.7 (m, 24F), −123.5 to −123.9 (m, 16F), −127.0 (m, 8F). ^{13}C NMR ($CDCl_3$) δ: 22.3, 29.9, 39.2, 66.9, 69.4, 69.5, 70.0, 70.1, 70.3, 70.6, 70.7, 128.2, 128.7, 128.8, 136.0, 136.1.

4.1.7. Calix[4]arene tetraphosphoric acid, 2. Benzyl protected phosphate **12** of 160 mg was dissolved in 15 mL absolute ethanol. Excess Pd/C was added and a hydrogen balloon was adapted to the flask. The hydrogenation was conducted overnight, after which the mixture was filtered through Celite and concentrated down under an air stream. White solid of 55 mg was obtained (41% yield). MALDI-FTMS $[M-H]^-$ calcd for $C_{112}H_{123}F_{68}N_4O_{36}P_4$ 3516.97, found 3516.547, $[M-Na]^-$ calcd for $C_{112}H_{116}F_{68}N_4Na_7O_{36}P_4$ 3670.83, found 3670.349. ^1H NMR (CD_3OD)

δ: 1.65–1.7 (br m, 8H), 1.9–2.1 (br m, 8H), 2.2–2.4 (br m, 8H), 3.1–3.25 (d, 4H), 3.4–3.7 (m, 64H), 3.9–4.0 (m, 8H), 4.5–4.65 (m, 4H), 4.95–5.1 (m, 16H), 6.4–6.6 (br s, 8H). ^{19}F NMR (CD_3OD) δ: −83.1 (t, 12F), −115.4 (m, 8F), −123.3 to −123.5 (m, 24F), −124.4 (m, 16F), −128.0 (m, 8F).

4.1.8. Calix[4]arene tetrakis(imino-diethylcarboxylate), 13. To an anhydrous tetrahydrofuran solution (5 mL) under an argon atmosphere of 5,11,17,23-tetra(heptadecafluoroundecyl)-25,26,27,28-calix[4]arene tetraacetic acid (168 mg, 0.067 mmol), 77.06 mg (0.402 mmol) 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), and 54.3 mg (0.402 mmol) 1-hydroxybenzotriazole hydrate (HOBt) were added, followed by anhydrous dimethylformamide dropwise until all the solids dissolved. After stirring at room temperature for 3 h, a dimethylformamide solution of 1-bis(ethoxycarbonylmethyl)amino-11-amino-3,6,9-trioxaundecane **10** (175.03 mg, 0.402 mmol) was added and stirring was continued at room temperature for 6 d. After removal of all solvents in vacuo, the residue was taken up in dichloromethane and purified by flash chromatography (5% methanol in dichloromethane) to give 159 mg product (60% yield). Final purification was carried out by preparative HPLC on a Jordi Gel DVB 500 Å column, using a gradient of 90–100% acetonitrile/water over 30 min (retention time=7–8 min). MALDI-FTMS $[M+Na]^+$ calcd for $C_{144}H_{172}F_{68}N_8O_{36}Na$ 3904.08, found 3904.448. ^{1}H NMR ($CDCl_3$) δ: 1.2–1.3 (t, 24H, J=7.2 Hz), 1.6–1.8 (br m, 8H), 1.9–2.1 (br m, 8H), 2.2–2.4 (br m, 4H), 2.9–3.0 (br m, 4H), 3.15–3.25 (d, 4H, J=14 Hz), 3.4–3.65 (m, 73H), 4.15–4.2 (q, 12H, J=7.2 Hz), 4.65–4.70 (br m, 12H), 6.2–6.6 (br s, 2H). ^{19}F NMR ($CDCl_3$) δ: −81.6 (t, 12F), −114.8 (m, 4F), −122.6 to −122.7 (m, 21F), −123.5 to −124.1 (m, 12F), −127.0 (s, 8F).

4.1.9. Calix[4]arene octacarboxylate, 3. Starting material of 43 mg (**3**–**30**) was dissolved in 2 mL methanol, 0.5 mL 2 M NaOH was added, followed by an additional 1.5 mL water to dissolve all the solids. The mixture was stirred at room temperature for 3 d. After removal of the methanol, the water was lyophilized off to give a white-yellow solid (yield >99%). The product was characterized by ^{1}H NMR and ^{19}F NMR in CD_3OD. The signals are broad, indicating self-assembly, but the peaks can be easily identified. Although the product is very soluble in D_2O, NMR could not be used for characterization due to the strong self-assembly process. For example, in the ^{19}F NMR in D_2O only one very broad peak was observed in the CF_3 region and no peaks in the CF_2 region. MALDI-FTMS for the free octacarboxylic acid: $[M+Na]^+$ calcd for $C_{128}H_{140}F_{68}N_8O_{36}Na$ 3681.40, found 3682.018. ^{1}H NMR (CD_3OD) δ: 1.2–1.4 (br m, 25H), 1.7–1.8 (br m, 5H), 1.9–2.2 (br m, 6H), 3.4–3.7 (m, 79H), 3.9–4.0 (m, 8H), 4.5–4.65 (br m, 16H), 6.4–6.6 (br s, 7H). ^{19}F NMR (CD_3OD) δ: −83.1 (t, 12F), −115.6 (m, 5F), −123.3 to −123.5 (m, 20F), −124.4 (m, 11F), −128.0 (m, 8F).

4.2. Dynamic light scattering (DLS)

DLS measurements were carried out at 20 and 25 °C using a Zeta Potential/Particle Sizer Nicomp™ 380 ZLS. The samples of calix[4]arene tetraphosphate **2** and calix[4]arene octacarboxylate **3** were prepared in aqueous buffers of different pH by sonication of a thin film, obtained by evaporation of a methanol solution. The final concentrations were 100 μM and all solutions were filtered through a 450 nm microfilter before the measurements. For each sample, DLS measurements were carried out for 5 cycles of 10 min each or 10 cycles of 5 min each and were proved to be reproducible. The viscosities and refractive indexes for the aqueous buffers were measured. A Cannon-Manning Semi-micro viscometer size 25 (range 0.5–2 cSt) was used, equilibrated at 25 °C in a water bath, to determine the kinematic viscosity as: kinematic viscosity (cSt)=time× 0.002029 cSt/s. The dynamic viscosity was obtained using the equation: dynamic viscosity (cP)=kinematic viscosity (cSt)×ρ (g/mL), where 0.00029 is the viscometer constant at 25 °C and ρ is the density of the buffer, which was measured by weighing 1 mL of buffer. Both the density and the viscosity measurements were done in triplicate and average values were used for further calculations.

4.3. Transmission electron microscopy (TEM)

Samples were prepared as described above for the DLS study deposited on pioloform powder grids, negatively stained with methylamine tungstate and analyzed on a Philips CM120 electron microscope operating at 80 kV.

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Supplementary data

Volume-weighed NICOMP distribution analysis for solutions of calix[4]arene tetraphosphate and calix[4]arene octacarboxylate. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.04.030.

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